IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	Hall, et al.
Serial No.:	09/904,923
Filed:	July 13, 2001
For:	Modified Viral Surface Proteins for Binding to Extracellular Matrix Components
Group:	1632
Examiner:	Shukla

Commissioner of Patents
Box 1450
Alexandria, VA 22313-1450

SIR:

In response to the Office Action dated August 26, 2003, Applicants submit a new sequence listing and amended Pages 5, 21, and 22 of the specification, which include amendments in which references to SEQ ID numbers have been added. No new matter has been added, and it is respectfully requested that the amended Pages 5, 21, and 22 be entered, and the sequence listing be inserted between the specification and claims.

A computer disc which includes the sequence listing, and a Statement Under 37 CFR 1.821(f), also accompany this response.

With respect to the reference to U.S. application Serial No. 08/837,223, in Applicants' Request for Filing a Continuation Application Under 37 CFR 1.53, Applicants requested that the specification be amended by inserting before the first line the sentence:

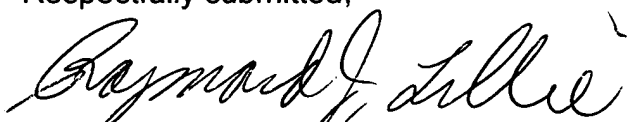
--This is a Continuation of Application Serial No. 08/837,223, filed April 10, 1997--.

Therefore, the above-identified application contains a reference to the parent application.

In response to the obviousness-type double-patenting rejection, Applicants submit with this response a terminal disclaimer, in which the terminal part of the statutory term of any patent granted on the above-identified application would not extend beyond the expiration date of U.S. Patent No. 6,004,798. It is therefore respectfully requested that the obviousness-type double-patenting rejection be reconsidered and withdrawn.

For the above reasons and others, this application is in condition for allowance, and it is therefore respectfully requested that the above-identified application be allowed.

Respectfully submitted,



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Box SEQ

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Patent Examining Operations

Application of:	Hall, et al.	Art Unit:	1632
Serial No.:	09/904,923	Examiner:	Shukla
Filed:	July 13, 2001		
Title:	Modified Viral Surface Proteins For Binding to Extracellular Matrix Components		
Attorney Docket No.:	271010-463	Customer No.:	27162

TRANSMITTAL LETTER

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

SIR:

Enclosed please find the following:

1. Response;
2. Sequence Listing;
3. Statement Under 37 C.F.R. 1.821 (f);
4. Computer Disc;
5. Terminal Disclaimer;
6. Corrected Pages 5, 21, and 22;
7. Petition for Extension of Time (1 Month);
8. Check in the amount of $240.00 –
9. A self-addressed, postage paid, return receipt postcard, date stamp and return of which is respectfully requested.

The Commissioner is authorized to charge payment of any additional filing fees required under 37 C.F.R. 1.16 associated with this communication or credit any overpayment to Deposit **Account No. 03-0678.**

FIRST CLASS CERTIFICATE

I hereby certify that this correspondence is being deposited today with the U.S. Postal Service as First Class Mail in an envelope addressed to:

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12/12/02

Raymond J. Lillie, Esq. **Date**

#204247 v1

Respectfully submitted,

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Figure 1A is a schematic of the receptor binding region of ecotropic gp70 protein (SEQ ID NO:1), showing the insertion of a polypeptide including a collagen-binding domain (SEQ ID NO:3) between amino acid residues 18 and 19;

Figure 1B is a schematic of the envelope structure and cloning strategy employed to insert a collagen-binding polypeptide (SEQ ID NO:3) flanked by linker amino acid residues into the unique BstEII site within the N-terminal region of ecotropic gp70 protein;

Figure 2A is a schematic diagram of the Moloney Murine Leukemia Virus envelope protein identifying the surface (SU) and transmembrane (TM) polypeptides, as well as the signal peptide, auxiliary collagen-binding domain, membrane spanning and R peptide regions;

Figure 2B shows an SDS-PAGE demonstrating the expression, purification, and renaturation of a chimeric envelope protein including a collagen-binding domain;

Figure 2C shows the binding of the renatured recombinant chimeric envelope protein in collagen-coated microtiter wells;

Figure 3A shows mock transfected (control) GPL cells that exhibit no positive staining for gp70 env protein;

Figure 3B shows GPL cells transfected with CEE+, which expresses wild-type gp70;

Figure 3C shows GPL cells transfected with chimeric ECB-CEE+ plasmid DNA;

Figure 3D is a Western Blot showing co-migration of the chimeric ECB-CEE+ env protein with wild-type CEE+ env protein, as well as co-migration of the gag proteins in the 30 kda region;

Figure 3E shows selective binding of chimeric viruses to collagen matrices in microtiter wells;

Figure 4A shows a cell culture plate showing positive staining for β-galactosidase in cultures transduced with viruses bearing the chimeric ECB-CEE+ envelope protein, and

i.e., the modified viral surface protein forms a portion of the liposome wall. Such proteoliposomes may be employed for gene transfer or for drug delivery to cells located at a site of an exposed extracellular matrix component.

EXAMPLES

The invention now will be described with respect to the following examples; however, the scope of the present invention is not intended to be limited thereby.

Example 1

CEE+ is a CMV-env expression vector constructed by digesting CEE (Morgan, et al., J. Virol., Vol. 67, No. 8, pgs. 4712-4721 (August 1993)) with HindIII and NotI, filling in the NotI site with a Klenow fragment, and ligating the CMV-env cassette into pBluescript II SK^+ (Stratagene, La Jolla, California) digested with SmaI and HindIII. PCR and recombinant DNA technologies then were employed to make the construct ECB-CEE+, which includes a polynucleotide encoding a chimeric Moloney Murine Leukemia Virus based gp70 envelope protein (SEQ ID NO:1) that incorporates a high-affinity collagen binding domain (SEQ ID NO:3) within its primary structure (Figure 1A). The modified collagen binding domain (SEQ ID NO:3) was derived from a functional domain within von Willebrand Factor involved in the recognition of exposed vascular collagen sequences. (Takagi, et al., Biochemistry, Vol. 32, pgs. 8530-8534 (1992); Tuan, et al., Conn. Tiss. Res., Vol. 34, pgs. 1-9 (1996)). ECB-CEE+ incorporates a polypeptide which includes the collagen binding decapeptide WREPSFMALS (SEQ ID NO:3) This construct was designed specifically for targeting a retrovirus to collagen exposed by injury, inflammation, disease, or reparative surgical procedures. The cysteine residue within the original von Willebrand Factor sequence was replaced conservatively by a methionine, in order that the collagen binding domain (SEQ ID NO:3) would not interfere with the elaborate disulfide bond formation required for the folding and/or renaturation of gp70.

Flanking linkers also were designed to include glycine residues to increase rotational flexibility and to minimize steric hindrances, while a histidine residue was included to promote an external conformation of the collagen binding domain. The complete 19 amino acid polypeptide insert, which includes the collagen binding decapeptide (SEQ ID NO:3), is shown in Figure 1A and Figure lB.

The construct ECBT-CEE+ includes the same components as ECB-CEE+ as well as a six amino acid residue putative thrombin cleavage site, which has the sequence LVPRGS (SEQ ID NO:4) between the collagen-binding domain and the remainder of the envelope protein.

ECB-CEE+ and ECBT-CEE+ were constructed using PCR and recombinant DNA technologies as mentioned above. The collagen binding decapeptide WREPSFMALS (SEQ ID NO:3) is encoded by the following polynucleotide: TGG CGC GAA CCG AGC TTC ATG GCT CTG AGC (SEQ ID NO: 5) . The following PCR primers in making ECB-CEE+ were employed.

Sense (CBD-S1):5′ -ATC ACC TGG GAG GTA ACC GGC CAT AGT TGG CGC-3′ (SEQ ID NO:6)

Antisense (CBD-aSl) :5′ -CG ATC TCC ATT GGT TAC CAA GCT AGC ACC GCT-3′ (SEQ ID NO:7)

CBD-S1 also was employed in making ECBT-CEE+, along with the following antisense primer CBDT-aS2:

5′-CG ATC TCC ATT GGT TAC CAA GCT GCC GCG CGG CAC CAG ACC GCT CAG AGC-3′ (SEQ ID NO:8)

Collagen binding domains with proper linkers were amplified by PCR using the primers CBDS1 and CBDaS1 or CBDS1 and CBDaS2, respectively (94^{0}C 1 mm, 55^{0}C 10 min, 72^{0}C 10 min., 35 cycles). The PCR bands then were digested with BstEII. Cee+ was digested with BstEII, followed by dephosphorylation of the linearized Cee+ vector. The digested PCR bands were ligated to the linearized Cee+ vector to form ECB-CEE+ and ECBT-CEE+. The proper orientations of the cDNA constructs were confirmed by sequence analysis.